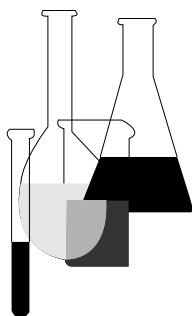




# Health Effects Test Guidelines OPPTS 870.6500 Schedule-Controlled Operant Behavior



## INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

**Final Guideline Release:** This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512-0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (<http://www.epa.gov/epahome/research.htm>) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

## **OPPTS 870.6500 Schedule-controlled operant behavior.**

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline are 40 CFR 798.6500 Schedule-Controlled Operant Behavior and OPP 85–5 Schedule-Controlled Operant Behavior (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals, Addendum 10, EPA report 540/09–91–123, March 1991).

(b) **Purpose.** In the assessment and evaluation of the potential human health effects of substances, it may be necessary to test for functional neurotoxic effects. Substances that have been observed to produce neurotoxic signs in other toxicity studies (e.g. central nervous system (CNS) depression or stimulation), as well as substances with a structural similarity to neurotoxicants affecting performance, learning, or memory may be appropriate to evaluate with this test. This guideline defines procedures for conducting studies of schedule-controlled operant behavior, one way of evaluating the rate and pattern of a class of learned behavior (under paragraphs (g)(1), (g)(4), (g)(5), and (g)(6) of this guideline. The purpose of the guideline is to evaluate the effects of acute and repeated exposures on the rate and pattern of responding under schedules of reinforcement. Any observed effects should be evaluated in the context of both the concordance between functional neurological and neuropathological effects and with respect to any other toxicological effects seen. Operant behavior tests may be also used to evaluate many other aspects of behavior (under paragraph (g)(3) of this guideline). Additional tests may be necessary to completely assess the effects of any substance on learning, memory, or behavioral performance.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this test guideline.

*Behavioral toxicity* is any adverse change in the functioning of the organism with respect to its environment in relation to exposure to a chemical substance.

*ED* is effective dose.

*Neurotoxicity* is any adverse effect on the structure or function of the nervous system related to exposure to a chemical substance.

*Operant, operant behavior, operant conditioning:* An operant is a class of behavioral responses which changes or operates on the environ-

ment in the same way. Operant behavior is further distinguished as behavior which is modified by its consequences. Operant conditioning is the experimental procedure used to modify some class of behavior by reinforcement or punishment.

*Schedule of reinforcement* specifies the relation between behavioral responses and the delivery of reinforcers, such as food or water (under paragraph (g)(2) of this guideline). For example, a fixed ratio (FR) schedule requires a fixed number of responses to produce a reinforcer (e.g. FR 30). Under a fixed interval (FI) schedule, the first response after a fixed period of time is reinforced (e.g. FI 5 min).

(d) **Principle of the test method.** Experimental animals are trained to perform under a schedule of reinforcement and measurements of their operant behavior are made. Several doses of the test substance are administered according to the experimental design (between groups or within subjects) and the duration of exposure (acute or repeated). Measurements of the operant behavior are repeated. For use of this test to study learning, animals may be trained following exposure. A descriptive and statistical evaluation of the data is made to evaluate the nature and extent of any changes in behavior in relation to exposures to the test substance. Comparisons are made between any exposures that influence the behavior and exposures that have neuropathological effects or effects on other targets of the chemical.

(e) **Test procedures—(1) Experimental design.** These test procedures may be used to evaluate the behavior of experimental animals receiving either acute or repeated exposures. For acute exposure studies, either within-subject or between-groups experimental designs may be used. For repeated exposure studies, between-groups designs should be used, but within-subject comparisons (preexposure and postexposure) are recommended and encouraged.

(2) **Animal selection—(i) Species.** For most studies the laboratory mouse or rat is recommended. Standard strains should be used. Under some circumstances other species may be recommended.

(ii) **Age.** Experimental animals should be young adults. Rats or mice should be at least 14 and 6 weeks old, respectively, prior to exposure.

(iii) **Sex.** Because of the labor-intensive nature of this testing, only one sex may be used. If data on the test chemical indicate that one sex is more sensitive to the test substance, or if it receives greater exposure, that sex should be used. If females are used, they should be virgins.

(iv) **Experimental history.** Animals should be experimentally and chemically naive.

(3) **Number of animals.** Eight to ten animals should be exposed to each level of the test substance and/or control procedure.

(4) **Control groups.** (i) A concurrent control group or control sessions (according to the design of the study) are required. For control groups, subjects should be treated in the same way as for an exposure group except that administration of the test substance is omitted.

(ii) Positive control data from the laboratory performing the testing should provide evidence that the experimental procedures are sensitive to substances known to affect operant behavior. Both increases and decreases in response rate should be demonstrated. Data based on acute exposures will be adequate. Permanently injurious substances need not be used. Historical data may be used if the essential aspects of the experimental procedure remain the same. Periodic updating of positive control data is recommended. New positive control data should also be collected when personnel or some other critical element in the testing laboratory has changed.

(5) **Dose levels and dose selection.** At least three doses should be used in addition to the vehicle control group (or sessions for within subject studies). Ideally, the data should be sufficient to produce a dose-effect curve. The Agency strongly encourage the use of equally spaced doses and a rationale for dose selection that will maximally support detection of dose-effect relations.

(i) **Acute studies.** The high dose need not be greater than 2 g/kg. Otherwise, the high dose should result in significant neurotoxic effects or other clearly toxic effects, but not result in an incidence of fatalities that would preclude a meaningful evaluation of the data. The middle and low doses should be fractions of the high dose. The lowest dose should produce minimal effects, e.g. an ED10, or alternatively, no effects.

(ii) **Subchronic (and chronic) studies.** The high dose need not be greater than 1 g/kg. Otherwise, the high dose should result in significant neurotoxic effects or other clearly toxic effects, but not produce an incidence of fatalities that would prevent a meaningful evaluation of the data. The middle and low doses should be fractions of the high dose. The lowest dose should produce minimal effects, e.g. an ED10, or alternatively, no effects.

(6) **Route of exposure.** Selection of route may be based on several criteria including, the most likely route of human exposure, bioavailability, the likelihood of observing effects, practical difficulties, and the likelihood of producing nonspecific effects. It should be recognized that for many materials more than one route of exposure may be important and that these criteria may conflict with one another. The route that best meets these criteria should be selected. Dietary feeding will be generally be acceptable for repeated exposure studies.

(7) **Combined protocol.** The tests described in this screening battery may be combined with any other toxicity study, as long as none of the requirements of either are violated by the combination.

(8) **Study conduct**—(i) **Apparatus.** Behavioral responses and the delivery of reinforcers should be controlled and monitored by automated equipment located so that its operation does not provide unintended cues or otherwise interfere with the ongoing behavior. Individual chambers should be sound attenuated to prevent disruptions of behavior by external noise. The response manipulanda, feeders, and any stimulus devices should be tested before each session; these devices should be calibrated periodically.

(ii) **Chamber assignment.** Concurrent treatment groups should be balanced across chambers. Each subject should be tested in the chamber to which it is initially assigned.

(iii) **Schedule of food availability.** (A) If a nonpreferred positive reinforcer is used, all subjects should be placed on a schedule of food availability until they reach a fixed percentage, e.g. 80 to 90 percent, of their ad libitum body weight, or kept at a fixed weight and fed after each session.

(B) Subjects must be trained until they display demonstrable stability in performance across days prior to exposure. One simple and useful criterion is a minimum number of sessions on the schedule and no systematic trend during the 5 days before exposure.

(iv) **Time, frequency, and duration of testing**—(A) **Time of testing.** All experimental animals should be tested at the same time of day and with respect to the time of exposure. For acute studies, testing should be performed when effects are estimated to peak, which may be estimated from data on the functional observational battery, motor activity, or from pilot studies. For subchronic studies, subjects should be tested prior to daily exposure in order to assess cumulative effects.

(B) **Frequency of testing.** The maintenance of stable operant behavior normally will require regular and frequent (e.g. 5 days a week) testing sessions. Animals should be weighed on each test day.

(C) **Duration of testing.** Experimental sessions should be long enough to reasonably see the effects of exposure, but brief enough to be practical. Under most circumstances, a session length of 30–40 min should be adequate.

(v) **Schedule selection.** The schedule of reinforcement chosen should generate response rates that may increase or decrease as a function of exposure. Many schedules of reinforcement can do this: A single schedule maintaining a moderate response rate, FI schedules, which engender a vari-

ety of response rates in each interval, or multiple schedules, where different components may maintain high and low response rates.

(f) **Data reporting and evaluation.** The final test report must include the following information:

(1) **Description of equipment and test methods.** (i) A description of the experimental chambers, programming equipment, data collection devices, and environmental test conditions should be provided. Procedures for calibrating devices should also be described.

(ii) A description of the experimental design including procedures for balancing treatment groups and the stability criterion should be provided.

(iii) Positive control data from the laboratory performing the test that demonstrates the sensitivity of the schedule used should be provided. Historical data may be used if all essential aspects of the experimental protocol are the same. Historical control data can be critical in the interpretation of study findings. The Agency encourages submission of such data to facilitate the rapid and complete review of the significance of effects seen.

(2) **Results.** Data for each animal should be arranged in tabular form by test group including the animal identification number, body weight, preexposure rate and patterns of responding, changes in response rate and patterns produced by the chemical, and group data for the same variables, including standard measures of central tendency and variability, e.g. means and standard deviations, and results of statistical analyses.

(3) **Evaluation of data.** (i) The findings should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlated functional and histopathological findings. The evaluation should include the relationship between the doses of the test substance and the incidence and magnitude of any observed effects, i.e. dose-effect curves for any effects seen.

(ii) The evaluation should include appropriate statistical analyses. Choice of analyses should consider tests appropriate to the experimental design, including repeated measures. There may be many acceptable ways to analyze the data.

(iii) Citations from the literature related to the interpretation of the neurotoxicity of the test material should also be included.

(g) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Dews, P.B. Assessing the Effects of Drugs, In *Methods in Psychobiology*, Vol. 2, (Ed.) R.D. Myers. Academic, NY. pp. 83–124 (1972).

(2) Ferster, C.B. and Skinner, B.F. *Schedules of Reinforcement*. Appleton-Century-Crofts, NY (1957).

(3) Laties, V.G. How Operant Conditioning Can Contribute to Behavioral Toxicology, *Environmental Health Perspectives* 28:29–35 (1978).

(4) National Academy of Sciences. *Principles for Evaluating Chemicals in the Environment*. National Academy of Sciences, Washington, DC (1975).

(5) National Academy of Sciences. *Principles and Procedures for Evaluating the Toxicity of Household Substances*. National Academy of Sciences, Washington, DC (1977).

(6) National Academy of Sciences. Strategies to determine needs and priorities for toxicity testing. Appendix 3B. *Reference Protocol Guidelines for Neurobehavioral Toxicity Tests* 2:123–129 (1982).